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HIV-1-Specific Chimeric Antigen Receptors Based on Broadly-Neutralizing Antibodies

(Running Title: Novel HIV-1-Specific bnAb CARs)

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ABSTRACT

Although the use chimeric antigen receptors (CARs) based on single chain antibodies for gene immunotherapy of cancers is increasing due to promising recent results, the earliest CAR therapeutic trials were for HIV-1 infection in the late 1990s. This approach utilized a CAR based on human CD4 as a binding domain, and was abandoned for lack of efficacy. The growing number of HIV-1 broadly neutralizing antibodies (bnAbs) offers the opportunity to generate novel CARs that may be more active and revisit this modality for HIV-1 immunotherapy. We used sequences from seven well-defined bnAbs varying in binding sites and generated single chain antibody-based CARs. These included 10E8, 3BNC117, PG9, PGT126, PGT128, VRC01, and X5. Each novel CAR exhibited conformationally relevant expression on the surface of transduced cells, mediated specific proliferation and killing in response to HIV-1-infected cells, and conferred potent antiviral activity (reduction of viral replication in $\log_{10}$ units) to transduced $CD8^+$ T lymphocytes. Their antiviral activity was reproducible but varied according to the strain of virus. These findings indicated that bnAbs are excellent candidates for developing novel CARs to consider in the immunotherapeutic treatment of HIV-1.

IMPORTANCE

While chimeric antigen receptors (CARs) using single chain antibodies as binding domains are growing in popularity for gene immunotherapy of cancers, the earliest human trials of CARs were for HIV-1 infection. However, those trials failed and the approach was abandoned for HIV-1. The only tested CAR against HIV-1 was based on using CD4 as the binding domain. The growing availability of HIV-1 broadly neutralizing antibodies (bnAbs) affords the opportunity to revisit gene immunotherapy for HIV-1 using novel CARs based on single chain antibodies. Here we construct and test a panel of seven novel CARs based on diverse bnAb types, and show that all are functional against HIV-1.

INTRODUCTION

Recent years have seen a surge in immunotherapeutic approaches for treating malignancy, including numerous promising human trials of chimeric antigen receptor (CAR) gene therapy to generate tumor-specific T cells, based on the importance of $CD8^+$ T lymphocytes (CTLs) in tumor surveillance and malignant cell clearance through cytotoxicity. The general approach has been to identify monoclonal antibodies that bind a tumor cell surface antigen, and use a single chain version of the antibody as an artificial T cell receptor by genetic fusion to the CD3 ζ chain signaling domain. As opposed to native T cell receptors (TCRs), CARs have the advantage of being MHC unrestricted and therefore broadly applicable across human individuals, and also unaffected by tumor cell immune evasion through MHC downregulation.

Notably, one of the earliest tested clinical applications of CARs was the treatment of HIV-1 infection. In 1994, Roberts *et al* designed two virus-specific CARs using CD4 or a single chain antibody as binding domains for recombinant gp120 on the surface of cells (1), and these were shown subsequently to have direct capacity to kill HIV-1-infected cells and suppress viral replication at levels similar to HIV-1-specific CTL clones isolated from infected persons (2). Based on these data, the CD4-based CAR, consisting of the CD4 extracellular and transmembrane domains fused to the CD3 ζ intracellular signaling domain (CD4- ζ), was advanced to clinical trials starting in the late 1990s, using retroviral transduction of autologous peripheral blood T lymphocytes and reinfusion. Unfortunately this effort was abandoned after these trials showed safety but no clear benefits; one study in viremic subjects showed no reduction in viremia although there appeared to be lowered rectal tissue virus burden (3), while

another in antiretroviral drug-treated subjects with baseline undetectable viremia showed not surprisingly no change in persisting blood viral reservoir in the form of proviral DNA (4). Follow-up of these studies after more than a decade did show low-level persistence of transduced cells without evidence of malignancy (5).

Several factors may have contributed to failure in these trials. The Moloney-based retroviral vector was relatively inefficient, and peripheral blood T cells were massively expanded *ex vivo* using supraphysiologic levels of interleukin-2, likely contributing to rapid loss of CAR expression and death of re-infused cells. The CAR itself may have been problematic; the CD4 domain may have allowed HIV-1 infection of transduced CTLs, or there could have been selection for viral escape through reduced CD4 binding, which can vary highly between different HIV-1 envelopes (6).

The identification of a growing number of broadly-neutralizing antibodies (bnAbs) against HIV-1 offers the possibility of creating new HIV-1-specific CARs with improved properties. These bnAbs have high affinity and excellent reactivity against varying HIV-1 strains, which could translate to efficient CARs with broad coverage of HIV-1 variation. Here we report the generation and testing of CARs based on seven bnAbs that recognize diverse epitopes on the HIV-1 envelope.

MATERIALS AND METHODS

Cells and media. The immortalized HIV-1-permissive CD4-expressing cell lines T1 (7), T2 (8), and Jurkat cells were maintained as previously described (9-11) in complete medium (R10) consisting of RPMI 1640 (Lonza, Allendale, NJ) supplemented with 2mM L-glutamine (Mediatech, Manassas, VA), 100 U/ml penicillin (Mediatech, Manassas, VA), 100 U/ml streptomycin (Mediatech, Manassas, VA), 10 mM HEPES (Sigma, St. Louis, MO), and 10% heat-inactivated fetal bovine serum (FBS) (Sigma, St. Louis, MO). 293T cells were maintained in Dulbecco's Modified Essential Medium supplemented with L-glutamine, penicillin, streptomycin, and FBS as above and previously described (12). Primary $CD8^+$ T lymphocytes were isolated from peripheral blood mononuclear cells (PBMCs) of healthy HIV-1-uninfected donors using anti-CD8 antibody coated magnetic beads as per manufacturer's directions (MACS column separation kit, Miltenyi, San Diego, CA) and then cultured for 5 days in R10 supplemented with 50 U/ml recombinant human interleukin-2 (NIH AIDS Reagent Repository) (R10-50) in the presence of the anti-CD3 antibody 12F6 (13), yielding purity of >99% $CD3^+/CD8^+$ cells by flow cytometry. All experiments were confirmed with cells from multiple donors and showed no significant donor-specific differences.

Construction of CAR vectors. The backbone for novel CAR constructions was the pTRPE-cMET-BBζ CAR plasmid provided as the generous gift of Dr. Carl June. This lentiviral expression vector (**Figure 1**) contained the gene for second generation CAR with a single chain antibody against hepatocyte growth factor receptor (cMET) fused to human IgG_4 hinge sequence, human CD8 transmembrane sequence, and cytoplasmic domains of human 4.1BB

(CD137) and human CD3 complex ζ chain (CD247). This vector was modified by creating a novel Apa I restriction site via a silent mutation in the hinge sequence (**Figure 1**). This was accomplished by subcloning the Xba I-Sma I restriction fragment into pUC19, in which the mutation was created by point mutagenesis (QuikChange kit, Invitrogen, Carlsbad, CA). After sequencing of the entire fragment to ensure no PCR-induced errors, this restriction fragment was ligated into the parental vector. Single chain antibody sequences of heavy chain-linker-light chain (**Table 1**) were synthesized as codon-optimized genes preceded by the signal sequence for granulocyte-macrophage colony stimulating factor (MLLLVTSLLLCELPHPAFLLIP) and followed by the beginning of the hinge region, flanked by Xba I and Apa I restriction sites, allowing ligation into the parental vector after restriction digestion. The bnAb sequences used in this approach included 10E8 (14), 3BNC117 (15), PG9 (16), PGT126 (17), PGT128 (17), VRC01 (18), and X5 (19).

Production of lentiviral vectors and transduction of cells. Lentivirus was produced by co-transfection of 293T cells with a CAR lentiviral vector construct plasmid (10μg) in conjunction with packaging and pseudotyping vectors including the lentiviral packaging plasmid pCMVDR8.2DVPR (7μg) and the vesicular stomatitis virus envelope glycoprotein G expression vector pHCMVG (3μg) using BioT transfection reagent (per the manufacturer's protocol, Bioland, Paramount, CA) with 5 x 10^6 293T cells that had been seeded in a T75 tissue culture flask 24 hours previously. Supernatants were obtained 24 and 48 hours after transfection, passed through a 0.45μm filter, and concentrated by ultracentrifugation (26,000 rpm for 90 minutes at 4˚C, SW28 rotor, Beckman Coulter, Fullerton, CA). Aliquots containing approximately 50ng HIV-1 p24 antigen in 50μl were frozen at -80˚C until use.

For transduction of primary $CD8^+$ T lymphocytes, polystyrene 6-well plates (BD Biosciences, San Jose, CA) were coated with RetroNectin according to the manufacturer's instructions (Takara, Mountain View, CA). An aliquot of lentiviral vector was diluted to 500µl in R10 and placed in a pre-coated well, followed by centrifugation at 2000g for 2 hours at 32˚C (Sorvall Legend RT, ThermoFisher Scientific, Grand Island, NY). After aspiration of the medium, 10^6 recently stimulated $CD8^+$ T lymphocytes were added per well in a total volume of 2ml R10-50. After overnight incubation in a tissue culture incubator, the cells were transferred to fresh R10-50 and cultured for about 7 days before assessment of transduction efficiency (below).

For Jurkat cells, 10^6 cells in log phase growth were incubated with the lentiviral vector for four hours with intermittent shaking, washed, and resuspended in fresh R10.

Western blotting for CD3 ζ. Cell lysate from 2 x 10^6 transduced cells was prepared by lysing the cells in lysis buffer (0.5 % NP-40, 0.5 % sodium deoxycholate, 50 mM NaCl, 25 mM Tris-HCl, 10 mM EDTA) containing 10 mM phenylmethyl sulfonyl (Sigma, St Louis, MO) and 1X HALT protease inhibitors (Invitrogen, Carlsbad, CA). Proteins were separated by loading 20 µl of the lysate onto a 10% NuPAGE Bis-Tris gel (Invitrogen, Carlsbad, CA) and electrophoresis, followed by blotting onto a 0.45 µM PVDF membrane (Millipore, Billerica, MA). The membrane was probed using a mouse anti-human CD247 monoclonal antibody (catalog #551033, BD Pharmingen, San Jose, CA) and the SuperSignal West Pico Detection Kit (Pierce, Rockford, IL).

Flow cytometry for cell surface single chain antibody expression. Transduced cells were washed, resuspended in 100 µl of wash buffer (5% BSA with 2mM EDTA in PBS) containing either FITC-conjugated goat anti-human F(ab)2 antibody (Cat. #109-006-003, Jackson ImmunoResearch Laboratories, West Grove, PA) or isotype control antibody and incubated for 30 minutes at 4˚C. After washing in fresh wash buffer, the cells were fixed in 1 % paraformaldehyde and analyzed by flow cytometry (LSR Fortessa II cytometer, BD Biosciences, and FlowJo software, Ashland, OR).

Flow cytometry for CAR-mediated proliferation of transduced CD8$^+$ T lymphocytes in response to HIV-1-infected target cells. HIV-1-infected T2 cells, which are MHC class I low due to a deletion in the transporter associated with processing (TAP) (8) and previously shown to be suitable target cells for an HIV-1-specific CAR (20), served as target cells. These were infected with an excess of HIV-1 NL4-3-based reporter virus containing a gene for murine CD24 (mCD24) in the vpr locus (21) to yield >90% infected cells by 3 or 4 days after infection, as previously reported (9, 10, 12). These were irradiated immediately before use with 10,000 rads in a cesium irradiator, as well as peripheral blood mononuclear cells from a healthy donor with 3,000 rads (feeder PBMCs). CAR-transduced primary CD8$^+$ T lymphocytes were labeled with CellTrace Violet and washed according to manufacturer's directions (ThermoFisher Scientific, Grand Island, NY). In a 48 well plate well, 5 x 10^5 labeled transduced cells were added to 5 x 10^5 irradiated infected T2 cells and 2 x 10^6 irradiated feeder PBMCs, and cultured in 1ml R10-50 for five days with a medium change after three days. Flow cytometry (LSR Fortessa II cytometer, BD Biosciences) was then performed with co-staining for human CD8 (PerCP-anti-human CD8,

195 catalog #30130, Biolegend, San Diego, CA) and analysis of proliferation using FlowJo software
196 (FlowJo, Ashland, OR).
197
198 **Chromium release killing assays for CAR-mediated killing of HIV-1-infected target cells.**
199 T2 cells infected with HIV-1 NL4-3 as above were used as target cells for the CAR-transduced
200 primary $CD8^+$ T lymphocytes in standard ^{51}Cr-release assays as previously described (9, 10, 12).
201 Briefly, infected and control uninfected T2 cells were ^{51}Cr-labeled for 1 hour and incubated with
202 or without effector $CD8^+$ T lymphocytes for 4 hours at varying cell ratios in a 96-well U-bottom
203 plate. Supernatants were then harvested for measurement of extracellular ^{51}Cr by micro-
204 scintillation counting in 96 well plates. Spontaneous release was measured on target cells without
205 effector cells, and maximal release was measured on target cells lysed with 2.5% Triton X-100.
206 Specific lysis was calculated as: (experimental released chromium - spontaneous release) ÷
207 (maximal release - spontaneous release).
208
209 **Virus suppression assays.** The ability of CAR-transduced $CD8^+$ T lymphocytes to suppress the
210 replication of HIV-1 was tested as previously described in detail (2, 9, 11, 12, 22-24). HIV-1
211 strains tested were obtained from the NIH AIDS Reference and Reagent including
212 94US_33931N (catalog# 11250), 90_US873 (catalog# 11251), 96TH_NP1538 (catalog# 11252),
213 00TZ_A246 (catalog# 11256). In brief, T1 cells transduced with human CCR5 were infected at
214 a multiplicity of 0.1 tissue culture infectious doses per cell, and co-cultured in a 96-well plate
215 with CAR-transduced cells at a ratio of 5×10^4 to 1.25×10^4 cells respectively in 200 µl of R10-
216 50, or no effector cells as a control. The effector cells had been confirmed to be >90%

transduced. Each condition was run in triplicate, and viral replication was monitored using p24 quantitative ELISA (XpressBio, Frederick, MD).

RESULTS

Genetic construction of chimeric antigen receptors (CARs) based on broadly-neutralizing antibodies (bnAbs) against HIV-1. A set of bnAbs was selected based on binding of different HIV-1 Env domains and availability of sequences (**Table 1**). These included seven antibodies targeting the CD4 binding site, the CD4 binding-induced site on gp120, the gp120 V2 loop, gp120 N-glycans, and the membrane proximal region of gp41. Genes for single chain versions of each antibody were created by synthesis of codon-optimized sequences for the heavy and light chains, separated by a GGGGSx3 linker, and these genes were substituted for the single chain antibody in a second generation CAR vector containing the 4-1BB signaling domain fused to the CD3 ζ signaling domain (**Figure 1**).

The novel CARs were confirmed for expression and potential functionality. The CAR genes were delivered by lentiviral vectors to Jurkat cells for initial confirmation of expression and functionality. Western blotting for CD3 ζ confirmed that the transduced cells contained both native CD3 ζ and the expected larger CD3 ζ-containing CAR for all seven constructs (**Figure 2**). Flow cytometry for cell surface CAR expression using a goat antibody against human F_{ab} (antigen-binding antibody fragment) further demonstrated cell surface expression of each CAR (**Figure 3**). Primary $CD8^+$ T lymphocytes were then transduced with the lentiviral vectors, and flow cytometry also confirmed cell surface CAR expression for each construct, although the transduction efficiency was lower than for Jurkat cells. Using the goat anti-human F_{ab} antibody as a stimulus, there was selective expansion and enrichment of the CAR-transduced cells within

the bulk population (**Figure 4**), indicating that cross-linking of CARs induced proliferation of the transduced cells analogous to anti-CD3 antibody induced proliferation of normal T lymphocytes.

CAR-transduced primary CD8$^+$ T lymphocytes proliferated in response to HIV-1-infected cells. Enriched CAR-transduced (≥90%) primary CD8$^+$ T lymphocyte effector cells were tested for their capacity to proliferate in response to HIV-1-infected cells. After co-culture with irradiated HIV-1_{NL4-3}-infected T2 cells or control uninfected T2 cells, all effector cells transduced with CARs exhibited HIV-1-specific proliferation to varying degrees (**Figure 5**). These results confirmed retained specificity of the single chain versions of the parental antibodies against HIV-1 envelope on the surface of infected cells.

All novel CARs mediated specific killing of HIV-1-infected target cells. The enriched CAR-transduced effector cells were tested for specific killing of HIV-1-infected CD4$^+$ lymphocytes. They were assayed in standard chromium release assays against HIV-1_{NL4-3}-infected T2 cells or control uninfected T2 cells (**Figure 6**). All CARs mediated substantial killing of infected versus uninfected target cells at effector:target ratios of 5:1, indicating specific targeting of HIV-1-infected cells.

All novel CARs exhibited antiviral activity. The enriched CAR-transduced effector cells were also tested for antiviral activity against infected CD4$^+$ cells. T2-CCR5 cells were infected with a panel of HIV-1 strains including primary R5-tropic isolates and cultured in the absence or presence of the CAR-transduced effector cells. Virus replication was assessed by measurement of p24 antigen between days 7 to 10 of culture. Suppression of replication was calculated as the

265 difference of $\log_{10}$ units of p24 between cultures without versus with effector cells, which was
266 then normalized as the ratio to total replication without effector cells (**Figure 7**). Across multiple
267 experiments, each CAR exhibited consistent levels of antiviral activity against five HIV-1
268 strains, including four subtype B and one subtype C (**Figure 8**). For this limited set of viruses,
269 some CARs such as the one based on PGT126 appeared to have broader coverage than others
270 such as the one based on 3BNC117.
271

DISCUSSION

Given the success of CARs for cancer immunotherapy and shortcomings of the prior attempt of this approach for HIV-1 treatment, revisiting CARs for HIV-1 infection is appropriate. We took advantage of the new generation of bnAbs, which are remarkable for their affinity, potency, and breadth of HIV-1 neutralization. These were engineered as single chain constructs to serve as binding domains for novel CARs.

Somewhat unexpectedly given the uncertain affinity of antibodies converted to single chain versions, all seven CAR constructs showed HIV-1-specific functional activity. Each demonstrated conformationally relevant cell surface expression (by binding of a goat anti-human F_{ab} antibody) as well as mediation of HIV-1-specific proliferation, killing, and suppression of viral replication. The generally high binding affinity of bnAbs may have afforded retention of enough affinity of the single chain versions to meet the much lower affinity requirement of T cell receptors for signaling. Thus, each CAR conferred the functional properties that are likely important for transduced cell expansion and clearance of HIV-1-infected cells *in vivo*.

Against HIV-1_{NL4-3}, these CARs had similar antiviral properties to the pre-clinical *in vitro* antiviral tests of the CD4- ζ CAR (2) that was previously advanced to clinical trials. Matching these novel CARs, that CAR mediated about 50-60% lysis of cells infected with HIV-1_{IIIB} (the source of the Env in HIV-1_{NL4-3}) and about 40-50% log efficiency virus suppression. In a separate earlier study, the CD4- ζ CAR also mediated HIV-1-specific proliferation of transduced T lymphocytes (1).

It is unclear what *in vitro* assays might predict superiority of one CAR over the other for use *in vivo*. While proliferation and antiviral activity are likely to be the critical activities for efficacy *in vivo*, our *in vitro* assays are semi-quantitative and conditions may not reflect those *in vivo*. Our data suggest that some bnAb-based CARs have broader activity against HIV-1 variability than others, although more testing will be required. Given an optimal range of affinity for CARs against their target proteins, where higher or lower levels of affinity yield inferior activity (25), it may be that CARs will differ in their persistence depending on their affinity for the specific HIV-1 Env sequence encountered *in vivo*. In this regard, the demonstration by Webb *et al* (26) that different bnAbs have highly variable neutralization curve slopes might suggest that bnAbs with a flatter slope would have a larger "sweet spot" of affinity across varying HIV-1 Envs. They reported that CD4-binding site (including VRC01 and 3BNC117) and V3-glycan (including PGT128) bnAbs exhibit steeper slopes, while V2-glycan (including PG9) and MPER (including 10E8) bnAbs exhibit flatter slopes. However, this appears to be contradicted by our observation that the PG9- and 10E8-based CARs seem to have *less* breadth of antiviral activity than the VRC01- and 3BNC117-based CARs, suggesting the influence of other potential factors such as preservation of affinity in single chain form and/or greater reserve of affinity. Further supporting this point, while X5 was originally considered broadly-neutralizing, it has relatively poor neutralizing breadth against varying HIV-1 isolates compared to the other antibodies tested here (27), yet demonstrates good breadth as a CAR. Regardless, because bnAbs seem to be escaped by HIV-1 Env variation in their original hosts (28-30), viral variability and escape may remain a barrier to therapeutic implementation of bnAb-based CARs.

In summary, we have demonstrated that seven bnAbs varying in epitope specificity all have activity as single chain HIV-1 receptors in CARs. All constructs have the ability to recognize infected cells for proliferation, killing, and suppression of viral replication, although they may vary in their breadth of HIV-1 sequence diversity coverage. Additional studies will be necessary to understand and assay the properties important for transduced cell proliferation and function for *in vivo* immunotherapies based on bnAb CARs.

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448 **FIGURE LEGENDS**

449

450 **Figure 1. CAR structure and construction strategy.** The parental vector contained a CAR
451 based on a single chain antibody. This vector was modified with a silent mutation to create an
452 Apa I site (GG**C**CC**T**→GG**G**CC**C**) in the hinge region of the CAR gene (within a sequence-
453 confirmed Xba I-Sma I intermediate plasmid vector). New CAR genes were generated by
454 synthesis of single chain antibody genes that were substituted into this vector via Xba I-Apa I
455 restriction fragments.

456

457 **Figure 2. Confirmation of novel CAR expression via western blotting of transduced Jurkat**
458 **cells.** Western blotting for CD3 ζ was performed on Jurkat cells after transduction with CAR-
459 expression lentiviral vectors. The open arrow indicates the expected size of the native CD3 ζ
460 chain, and the closed arrow indicates the approximate expected size of the CAR (including the
461 single chain antibody, hinge, 4-1BB signaling, and CD3 ζ signaling domains). Lane M: Marker.
462 Lanes 1-8: CAR-10E8, CAR-3BN117, CAR-PG9, CAR-PGT126, CAR-PGT128, CAR-VRC01,
463 CAR-X5, and non-transduced Jurkat control respectively.

464

465 **Figure 3. Confirmation of novel CAR expression via flow cytometry for cell surface**
466 **immunoglobulin of transduced Jurkat cells.** Transduced Jurkat cells were stained with goat
467 antibody against-Human F_{ab} and assessed by flow cytometry. Histogram negative gating was set
468 on non-transduced control cells (not shown).

469

Figure 4. Selective functional expansion by binding of the single chain antibody domain of the novel CARs. After transduction of primary $CD8^+$ T lymphocytes with each of the seven CAR vectors, the cells were serially passaged (10 days each passage) using stimulation with a goat anti-Human Fab antibody with irradiated allogeneic feeder PBMC and IL-2. The percentage of cells determined to express CAR was determined by flow cytometry as in Figure 3.

Figure 5. Proliferation mediated by novel CAR interaction with HIV-1-infected target cells. Primary $CD8^+$ T lymphocytes transduced with the panel of CARs were enriched to >90% purity and labeled with CellTrace Violet, then co-cultured with irradiated HIV-1 NL4-3-infected T2 cells. CellTrace Violet fluorescence was assessed by flow cytometry after 7 days. The open histograms indicate transduced cells exposed to control uninfected cells, while the shaded histograms indicated those exposed to the infected cells.

Figure 6. Specific killing of HIV-1-infected target cells mediated by novel CARs. CAR-transduced primary $CD8^+$ T lymphocytes were co-cultured with HIV-1-infected T2 cells in standard four-hour chromium release assays to assess killing mediated by the CARs . PGT128- and PG9- based CARs were tested for killing in a separate experiment from the other CARs. The relative efficiencies of the CARs varied between experiments and no single CAR was consistently superior.

Figure 7. Sample calculation of % efficiency log suppression by CAR-transduced cells. T1-CCR5 cells were infected with the indicated viruses at multiplicity of infection 10^{-1} $TCID_{50}$/cell

and cultured with or without CAR 10E8-transduced $CD8^+$ T cells (>90% enriched) at an effector:target ratio of 1:4. Left: HIV-1 p24 antigen was measured by ELISA on day 7; log units of p24 antigen (log_{10} pg/ml) are indicated above each bar. Virus suppression by CAR-transduced cells ranged from 5.44-4.73 = 0.71 to 5.77-3.52 = 2.25 log_{10} units (80.5% to 99.4%) for HIV-1_{33931N} and HIV-1_{NL4-3} respectively. In general, replication without effector cells reached 3 to 6 log_{10} pg/ml units (10^3 to 10^6 pg/ml). Right: Virus suppression is normalized to total replication without effector cells as the percentage reduction in log_{10} units of p24 antigen comparing cultures with and without added effector cells:

For HIV-1_{NL4-3}: (5.77-3.52) ÷ 5.77 = 0.390 = 39.0%

For HIV-1_{873}: (5.06-3.77) ÷ 5.06 = 0.255 = 25.5%

For HIV-1_{33931N}: (5.44-4.73) ÷ 5.44 = 0.130 = 13.0%

Figure 8. Efficiencies of novel CAR-transduced primary $CD8^+$ T cells against a panel of HIV-1 isolates. CAR-transduced primary $CD8^+$ T cells were tested against a panel of 4 subtype B viruses and one subtype C virus (TZA246) to determine % efficiency log suppression, as shown in Figure 7. For each virus, bars represent the median of all replicates across 1-6 (mean 2.9) independent experiments each with triplicates, with standard error bars. Note that 30% efficiency log suppression for a typical experiment with control viral replication of 5 log_{10} units pg/ml would correspond to 1.5 log_{10} units reduction, or 96.8% suppression of viral replication.

513 **TABLES**

514

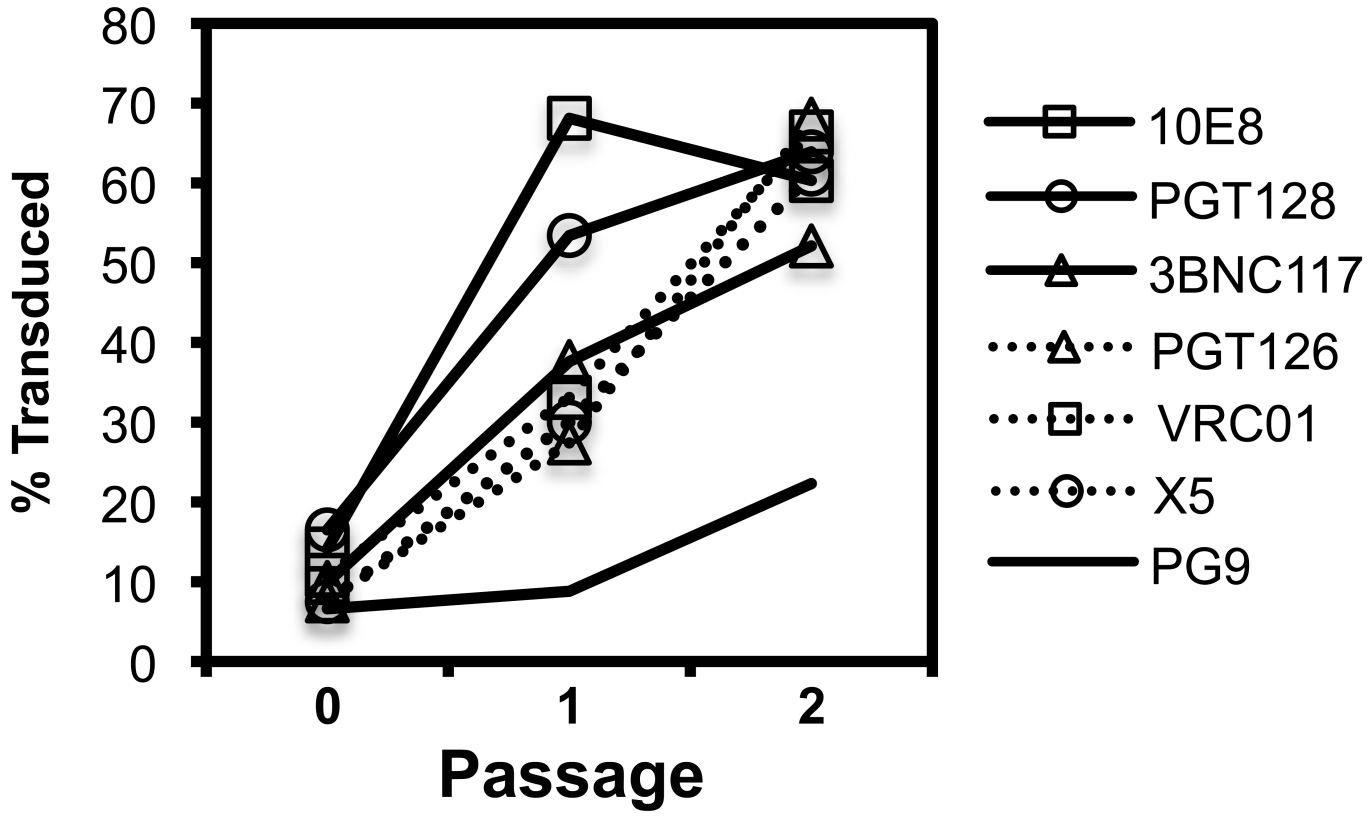
SPECIFICITY	AB		SEQUENCE
CD4 Binding Site	VRC01	VL	EIVLTQSPGTLSLSPGETAIISCRTSQYGSLAWYQQRPGQAPRLVIYSGSTRAAGIPDRFSGSRWGPDYNLTISNLESGDFGVYYCQQYEPFGQGTKVQVDIKR
		VH	MLLLVTSLLLCELPHPAFLLIPQVQLVQSGGQMKKPGESMRISCRASGYEFIDCTLNWIRLAPGKRPEWMGWLKPRGGAVNYARPLQGRVTMTRDVYSDTAFLELRSLTV DDTAVYFCTRGKNCDYNWDFEHWGRGTPVIVSS
	3BNC117	VL	DIQMTQSPSSLSASVGDTVTITCQANGYLNWYQQRRGKAPKLLIYDGSKLERGVPSRFSGRRWGQEYNLTINNLQPEDIATYFCQVYEFVVPGTRLDLKRTVAAP
		VH	MLLLVTSLLLCELPHPAFLLIPQVQLLQSGAAVTKPGASVRVSCEASGYNIRDYFIHWWRQAPGQGLQWVGWINPKTGQPNNPRQFQGRVSLTRHASWDFDTFSFYMDLK ALRSDDTAVYFCARQRSDYWDFDVWGSGTQVTVSSASTKGP
CD4-Induced Site	X5	VL	ELVLTQSPGTLSLSAGERATLSCRASQSVSSGSLAWYQQKPGQAPRLLIYGASTRATGIPDRFSGSGSGTDFTLTIGRLEPEDLAVYYCQQYGTSPYTFGQGTKLEI
		VH	MLLLVTSLLLCELPHPAFLLIPLEQSGAEVKKPGSSVQVSCKASGGTFSMYGFNWVRQAPGHGLEWMGGIIPIFGTSNYAQKFRGRVTFTADQATSTAYMELTNLRSDDT AVYYCARDFGPDWEDGDSYDGSGRGFFDFWGQGTLVTVSS
N-Glycan	PGT126	VL	QSALTQPPSASGSPGQSISISCTGTSNRFVSWYQQHPGKAPKLVIYGVNKRPSGVPDRFSGSKSGNTASLTVSGLQTDDEAVYYCSSLVGNWDVIFGGGTKLTVL
		VH	MLLLVTSLLLCELPHPAFLLIPQPQLQESGPGLVEASETLSLTCTVSGDSTAACDYFWGWVRQPPGKGLEWIGGLSHCAGYYNTGWTYHNPSLKSRLTISLDTPKNQVFL KLNSVTAADTAIYYCARFDGEVLVYHDWPKPAWVDLWGRGTLVTVTVSS
	PGT128	VL	QSALTQPPSASGSPGQSITISCTGTSNNFVSWYQQHAGKAPKLVIYDVNKRPSGVPDRFSGSKSGNTASLTVSGLQTDDEAVYYCGSLVGNWDVIFGGGTKLTVL
		VH	MLLLVTSLLLCELPHPAFLLIPQPQLQESGPTLVEASETLSLTCAVSGDSTAACNSFWGWVRQPPGKGLEWVGSLSHCASYWNRGWTYHNPSLKSRLTLALDTPKNLVFL KLNSVTAADTATYYCARFGGEVLRYTDWPKPAWVDLWGRGTLVTVSS
V2 Loop	PG9	VL	QSALTQPASVSGSPGQSITISCNGTSNDVGGYESVSWYQQHPGKAPKVVIYDVSKRPSGVSNRFSGSKSGNTASLTISGLQAEDEGDYYCKSLTSTRRRVFGTGTKLTVL
		VH	MLLLVTSLLLCELPHPAFLLIPQRLVESGGGVVQPGSSLRLSCAASGFDFSRQGMHWVRQAPGQGLEWVAFIKYDGSEKYHADSVWGRLSISRDNSKDTLYLQMNSLRVE DTATYFCVREAGGPDYRNGYNYYDFYDGYYNYHYMDVWGKGTTVTVSS
MPER	10E8	VL	SYELTQETGVSVALGRTVTITCRGDSLRSHYASWYQKKPGQAPILLFYGKNNRPSGVPDRFSGSASGNRASLTISGAQAEDDAEYYCSSRDKSGSRLSVFGGGTKLTVL
		VH	MLLLVTSLLLCELPHPAFLLIPEVQLVESGGGLVKPGGSLRLSCSASGFDFDNAWMTWVRQPPGKGLEWVGRITGPGEGWSVDYAAPVEGRFTISRLNSINFLYLEMNNL RMEDSGLYFCARTGKYYDFWSGYPPGEEYFQDWGRGTLVTVSS

515

516 **Table 1. Broadly neutralizing antibody sequences used for single-chain antibody CAR**

517 **constructions.** For each broadly neutralizing antibody, the specificity and heavy/light chain

518 amino acid sequences are given.



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